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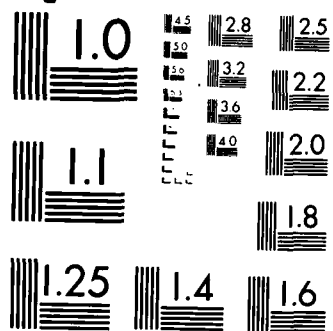
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CHEMOTHERAPY OF LEISHMANIASIS

Final Summary Report

by

Wallace Peters, MD, DSc.*

October 1979

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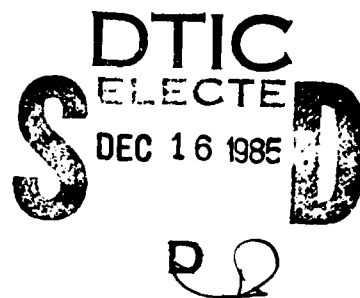
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) In the absence of almost any knowledge on the mode of action of currently available antileishmanial drugs, and on the ability or otherwise of the parasites to develop drug resistance, a project was initiated in Liverpool prior to our receiving Army support to attempt to explore these questions. The present report is an attempt to summarise the aims, progress and future plans of the writer on the subject of the chemotherapy of leishmaniasis.			

INTRODUCTION

Work carried out in Liverpool since the initiation of the first grant in April 1974 has been reported regularly in a series of seven documents to the Department of the Army. The present report is an attempt to summarise the aims, progress and future plans of the writer on the subject of the chemotherapy of leishmaniasis. Work ceased in Liverpool in September 1979 when the last grant period was concluded on the transfer of the writer to London. Steps are currently being taken to establish laboratories in the London School of Hygiene and Tropical Medicine (or its field station at Winches Farm, St. Albans) in which research in this area can be extended, and where leads exposed during the past five years can be followed up.

SCIENTIFIC ACTIVITIES

1. BASIC QUESTIONS INVESTIGATED

The fundamental postulates on which this programme was founded were as follows:- (i) The leishmaniasis pose a significant health problem in many tropical and subtropical regions. This problem is of importance to both civilian and military health services.

(ii) Preventive measures are not available or are of limited value for the leishmaniasis especially in areas where the diseases are essentially zoonotic.

(iii) Therapy of established infections depends on a small number of toxic drugs. Even these are not always curative.

(iv) Certain cases of leishmaniasis fail to respond to any known drug and may culminate in severely mutilating mucocutaneous lesions, in a diffuse cutaneous disease resembling lepromatous leprosy, or in a fatal visceral disease. The latter may also affect domestic dogs.

In the absence of almost any knowledge on the mode of action of currently available antileishmanial drugs, and on the ability or otherwise of the parasites to develop drug resistance, a project was initiated in Liverpool prior to our receiving Army support to attempt to explore these questions.

1.1. Chemotherapy

1.1.1. Tissue culture models

Even our best drugs, e.g. organic antimonials such as sodium stibogluconate (Pentostam) or meglumine antimoniate (Glucantime), fail completely to cure certain individuals infected with types of *Leishmania* that would be expected to produce simple, even self-healing lesions. This observation led us to establish a system for the comparison of the baseline drug sensitivity of parasites isolated from different patients, "responders" and "non-responders" in identical geographical areas. We were guided by two problematic assumptions (based on the literature), firstly that

the host's immune response would influence the activity of a specific drug against infection in vivo and, secondly, that the promastigote stages of Leishmania in cell-free cultures would respond differently from the amastigote stages that infect mammalian cells. In order to avoid these problems we decided to adopt infections in tissue culture rather than simple promastigote cultures of in vivo models. The two types of culture that proved most useful were the Sticker dog sarcoma, and mouse peritoneal macrophages. While we failed to grow all the then available parasites in these systems, we were able to cultivate on a regular basis a line of Leishmania mexicana mexicana which proved invaluable for our early studies on drug sensitivity. A series of papers was published describing this phase of the work and indicating a number of chemical types that appeared worth following up in vivo. (1,2,3)

1.1.2. Animal models

The only readily available animal models in which to study drug action when we started were the golden hamster, which had long been used as a host for L.donovani, and the albino mouse which was used as a model for L. tropica. Two circumstances influenced our subsequent search for other models, firstly the clear demonstration of the importance of the genetic background of the host in its response to infection with any given parasite and, secondly, the parallel programme that we evolved using biochemical taxonomy to pinpoint the precise nature of the lines of parasites with which we were working. (see 1.2).

After several years' experience in the development of different animal models we finally settled on the following lines of mice as hosts for three parasites, L.donovani sensu lato in NMRI mice, L.major (= L.tropica major) in random-bred albino mice, and L.m.amazonensis, also in random-bred albinos. (4,5) We have not yet succeeded in adapting L.panamensis or L.braziliensis into any line of mouse, rat or other readily available laboratory host other than the hamster, even using naturally or artificially immuno-deprived animals. The response of a large number of compounds, including many received for this purpose from WRAIR, have now been compared in vivo in these models and reports on this work are "in press". (6,7) Important leads that have arisen from this programme were reviewed in the September 1979 Final Report. We were able to confirm the high level of activity of a series of 8-aminoquinolines reported by other workers to be very active against L.donovani in the hamster. We also initiated our own studies on the incorporation of antimonials into liposomes (3), unaware of the work being carried out simultaneously by our colleagues in WRAIR on the same lines. The results obtained by WRAIR, ourselves and a third group at the Nuffield Institute in London were equally promising.

1.1.3 Mode of drug action

One of the first observations that we made in our tissue cultures was that none of the drugs known to be effective in man proved to have significant antiparasitic activity except at phenomenally high concentrations (2), although we did identify other groups of compounds that showed promise (3). This suggested several possibilities such as:-

- (i) the drugs required to be metabolised in vivo to form active metabolites
- (ii) drug action was based on an interaction between drug and host immunity
- (iii) our model was inappropriate.

The difficulty we were faced with was compounded by a lack of knowledge of the cellular basis of parasite immunity to the destructive activity of the host macrophages on the one hand, and the host's development of the ability to destroy parasites on the other. Moreover our knowledge of the fundamental metabolic processes of the amastigotes was also abysmally lacking. In order to understand how drugs function it was evidently necessary to study these processes in depth, and then attempt to identify points at which drugs might interact with them. Consequently we established several lines of investigation.

1.1.3.1. Study of the host-macrophage interaction

Preliminary experiments with L.m.mexicana demonstrated that amastigotes of this parasite were not destroyed by the peritoneal macrophages of mice that had had no previous exposure to them in vivo. On the contrary, macrophages removed from mice that had experienced previous exposure to the homologous organism did have the ability to destroy intracellular amastigotes in tissue culture.(9) Lymphocytes in the culture were found to be necessary for this process, and subsequent experiments showed that the macrophages required contact with the activated lymphocytes prior to their being invaded by the parasites.

Using classical lysosome labelling techniques at the ultrastructural level we then examined the relationship of host cell lysosomes in activated and non-activated macrophages to the parasitophorous vacuoles in which the amastigotes develop. We were able to demonstrate that the lysosomes fuse with the contents of the parasitophorous vacuoles but, in non-activated macrophages, the parasites retain their integrity. On the contrary, in activated macrophages the fusion of the lysosomes with the parasitophorous vacuoles seemed to lead to the destruction of the contained parasites.

Current studies are being directed to the elucidation of the mechanisms by which the parasites are apparently able to overcome the effect of the lysosomal enzymes with which they would appear to be surrounded. Azo-dye coupling procedures at the light microscope level indicated that the activity of one of these enzymes, acid phosphatase, was of an unusually low order, implying that the parasites are indeed capable of inactivating this enzyme at least. (10)

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1.1.3.2. The intracellular localisation and mode of action of antimony

Preliminary ultrastructural studies on the morphological changes produced in L.donovani amastigotes in the liver of mice treated with Pentostam indicated a significant decrease in the density of cytoplasmic ribosomes within the parasites. Evidence was also found of damage to the cytoplasm of the host cells in close proximity to the parasitophorous vacuoles, those containing apparently normal amastigotes as well as others with a decreased ribosomal complement. These observations suggest that antimony causes damage to the parasites not only directly, but perhaps also by adversely affecting the cytoplasm of host cells within their immediate vicinity. (11)

Work is currently in hand in Liverpool to identify the sites of localisation of radioactive pentavalent antimony using various techniques including autoradiography, and X-ray microanalytical procedures. It is hoped to transfer this project to London in the near future.

1.1.3.3. Biochemical studies in drug action

One of the major hurdles to be overcome in order to investigate the biochemical pathways of the amastigotes was to develop a reliable system for separating amastigotes from their host cells in bulk. A suitable technique developed by one of our research students has proved invaluable and has permitted him to make several important observations. (12) For example it appears that amastigotes of L.m.amazonensis in short-term extracellular cultures do not utilise glucose as their main source of energy. Nevertheless it was confirmed that they do possess some of the glycolytic enzymes such as GPI, G6PD, MDH and IDH. The amastigotes readily incorporate adenosine and uridine but not thymidine or orotic acid into nucleic acid. On the contrary, thymidine is incorporated by promastigotes.

Pentamidine at 10^{-5} M was shown to reduce adenosine uptake by the promastigotes but had no influence on amastigotes. A difference was also found in the sensitivity to this drug of the promastigotes of different lines of Leishmania, L.donovani LV9 and L.m.mexicana LV4 proving more sensitive than L.m.amazonensis. One result of exposure of the amastigotes to pentamidine appears to be an inhibition of their transformation to amastigotes. Ultrastructural examination of treated parasites showed extensive vacuolisation of the mitochondrion-kinetoplast region, suggesting that this diamidine may have a similar mode of action in Leishmania to that already well documented in other trypanosomatids, namely interaction with kinetoplast DNA.

Pentostam was shown to be taken up to a slight degree only by both amastigotes and promastigotes of L.m.amazonensis LV78. Even when incubated in a concentration of 10^{-3} M for 48 hours the parasites apparently remained alive and well, thus confirming our original observations with amastigotes inside Sticker sarcoma cells and isolated, unactivated mouse peritoneal macrophages.

1.2. Biochemical taxonomy

Early in our programme it became apparent that a number of parasites that we received with various labels such as "L.enriettii", "L.mexicana", "L.tropica" and so on were behaving in quite unpredictable fashion and we were faced with the question of just what was their identity. Since classical morphology is of little value in identifying the species of this genus, much less subspecies, we were obliged to turn to alternative methods using intrinsic properties of these organisms such as the DNA buoyant density and isoenzyme characterisation, as well as such extrinsic features as growth in different culture media, dispersal and rate of growth in vivo, etc.

This programme evolved into a major undertaking. We were able to utilise both nuclear and kinetoplast DNA characters (13), a battery of parasite enzymes (14), and the "Excretory Factor" serotypes previously described by Israeli workers. Over the years we built up an extensive reference collection of leishmanial isolates which now numbers over 700 and includes material from all endemic areas. A number of papers have been published which throw light on the epidemiology of the leishmaniasis. We were able to demonstrate the affinity, if not identity, for example, of an autochthonous Texan isolate from patients with cutaneous disease to Venezuelan isolates of L.mexicana pifanoi, of recent Indian visceral isolates to Mediterranean L. infantum (15) and the overall similarity of L. tropica from man, rodents and sandflies covering a very wide geographical area extending from the USSR to South of the Sahara. (16) Isolates from patients with drug responsive L.aethiopica were shown to be identical to others from "non-responders" in the same location (17) thus adding weight to the hypothesis that diffuse cutaneous leishmaniasis is due to a failure of host immunity and not to a difference in the infecting organism.

It is obvious that the precise pinpointing of the taxonomic identity of Leishmania that we can now perform is fundamental to all future studies on the chemotherapy, immunology and epidemiology of the leishmaniasis. (18) Other centres have now taken up our procedures and the development work involved is currently being extended by the writer's group in London. The Liverpool centre has been designated as a WHO Reference Centre for leishmanial identification.

1.3. Immunology

Although not directly supported by Army funds we have been conducting in parallel a number of studies on the nature of the host response to infection with Leishmania in addition to the investigation of the cellular mechanisms referred to above. These include the development of the ELISA test as a diagnostic tool for visceral leishmaniasis which has proved more sensitive than immunofluorescence. (19) We have studied both the humoral and cellular responses to cutaneous infection with different species of Leishmania in man, and have produced a technique for the demonstration of leishmanial antigen in standard, formalin-fixed blocks prepared for routine histology.

1.4. Other studies

Other projects carried out in Liverpool included the study in depth of virus-like particles discovered in L.hertigi (20, 21), the action of immunosuppressive and immunopotentiating drugs on the evolution of various leishmanial infections in vivo, an ultrastructural investigation of the Noguchi-Adler body, and a study of the effect of in vitro exposure of amastigotes to drugs prior to their inoculation into animals.

1. PROPOSALS FOR FUTURE WORK

The past five years have clearly been very fruitful and we have been able to make significant contributions of a practical nature in several areas. New avenues have been opened for the investigation of new antileishmanial drugs, and several promising types of compounds have been identified.

Now that the Liverpool programme has been reduced, our activities in the field of chemotherapy will be established in London. The first requirements are:-

- (i) the establishment of our in vivo models for the evaluation of drug action against L.tropica, L.mexicana and "L.donovani"
- (ii) the completion of work commenced in Liverpool to compare the action of a broad spectrum of compounds (mainly from WRAIR) against these three species

This will be followed by:-

- (iii) evaluation of activity of new compounds provided by WRAIR
- (iv) a comparison of the response to specified drugs of different isolates of the same parasite to confirm whether drug resistance is a phenomenon that occurs in Nature
- (v) the induction of resistance to certain standard drugs in order to forecast its potential as a hazard in Nature
- (vi) broadening of our studies on the biochemistry of the amastigotes, with special reference to
- (vii) the investigation of the mode of action of old and new drugs
- (viii) a study of the mechanisms involved in the development of drug resistance
- (ix) investigation of ways of preventing or overcoming drug resistance
- (x) a study of the genetic basis of drug resistance
- (xi) further attempts to produce in vivo models for L.panamensis and L.braziliensis.

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